Efficient and Selective Syntheses of *S*-Acyl and *N*-Acyl Glutathiones

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Received 8 February 2010

Abstract: Selective syntheses of *S*-acyl glutathiones are achieved in 79–98% yields using 1-acyl-1*H*-benzotriazoles in the presence of potassium bicarbonate in aqueous methanol at 20 °C. N-Acylation of *S*-(*p*-nitrobenzoyl) glutathione with 1-acyl-1*H*-benzotriazoles followed by deprotection of the *p*-nitrobenzoyl groups under mild conditions gave 63–78% yields of *N*-acyl glutathiones. These methodologies should be useful for the S-acylation and N-acylation of peptides and glycopeptides.

Key words: amino acids, acylation, glutathione, peptides, drugs

Glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH, **1**), a tripeptide derived from glycine, cysteine, and glutamic acid, is critical to a variety of cellular functions.[1] GSH, which possesses a γ-glutamyl linkage and a sulfhydryl group as two characteristic structural features, plays an important role in the human body antioxidant defense system. In its reduced form, GSH (**1**) is the most abundant nonprotein thiol in eukaryotic cells, and is involved in various physiological and metabolic functions of all mammalian cells.[2] The N- and S-blocked GSH derivatives inhibit mammalian glyoxalase I from human erythrocytes.[3]

S-Acetyl glutathione can act as selective apoptosis-inducing agent in cancer therapy[4] and as an antiviral agent against herpes simplex virus-1 infection.[5] *S*-Acetyl glutathione is also a potent antioxidant, providing a rationale for its combination with antiretroviral drugs.[6]

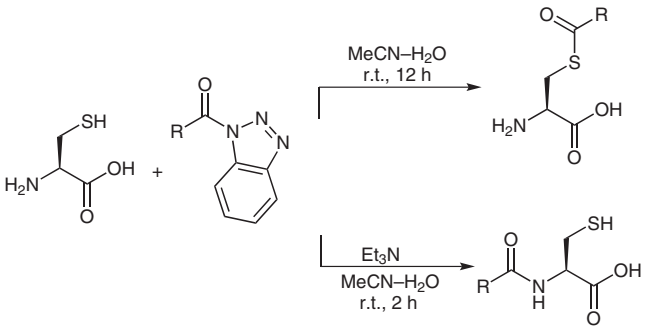
The hepatotoxic nonsteroidal anti-inflammatory drug Zomepirac, containing a carboxylic acid group, undergoes metabolic activation in vivo in rats, and in vitro in rat hepatocytes, to form unstable acyl-linked metabolites that react with GSH by transacylation forming the *S*-acyl glutathione conjugate of the drug. Therefore, the detection of *S*-acyl-linked GSH adducts of carboxylic acid drugs can be used as an indirect marker of drug bioactivation to chemically reactive transacylating metabolites.[7]

N-(4-Benzoylbenzoyl) glutathione is a photoaffinity label of glutathione *S*-transferases.[2] *N*-Acyl glutathiones bearing an aryl azide moiety provide photoactivatable analogues of GSH.[8]

Several literature reports of the selective S-acylation of GSH utilize (a) coupling reagents such as ethyl chloroformate–Et_3N[9] (b) reactions of GSH with acyl chlorides or anhydrides in trifluoroacetic acid,[10] and (c) use of enzyme.[11]

Published methods to synthesize *N*-acyl glutathiones have utilized (a) potassium phosphate buffer, which provided a mixture of N- and S-acylated products;[12] (b) an activated ester,[13] but Bernardi et al. observed only S-acylation of GSH using these conditions;[2] (c) N-acylation of GSSG with activated ester followed by reduction,[2,8,14] but this method fails when other susceptible groups like azide present in the molecule during the reduction;[8] (d) use of trityl group to protect thiol group of GSH then N-acylation followed by trityl group deprotection,[8] but overall yields are very low. Some of these methods also suffer from long reactions times (48 h).[2,8,14]

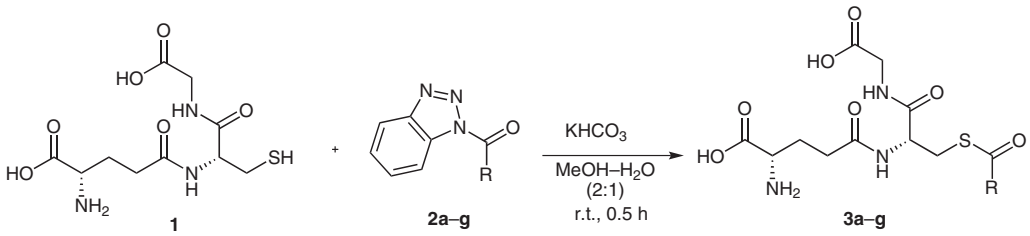
Recently, we have reported selective syntheses of *S*-acyl- and *N*-acylcysteines (Scheme 1).[15] However, our attempts to synthesize *S*-acyl glutathiones according to the above method for S-acylation of cysteine[15] failed to proceed after several days. Furthermore, treatment of GSH with *N*-acylbenzotriazoles in the presence of triethylamine did not yield *N*-acyl glutathiones, according to the method used for *N*-acylcysteines.[15] Use of excess of triethylamine gave low yields of S,N-diacylated product. Hence, the methods successful for the selective syntheses of *S*-acyl- and *N*-acylcysteines can not be used for selective synthesis of *S*-acyl and *N*-acyl glutathiones.



Scheme 1 Selective synthesis of *S*-acyl and *N*-acylcysteines

We now report facile, mild, and efficient syntheses of *S*-acyl and *N*-acyl glutathiones, which should have general applicability for S-acylation and N-acylation of biologically important larger peptides and glycopeptides. 1-Acyl-1*H*-benzotriazoles are stable crystalline compounds and advantageous for N-, O-, C-, and S-acylation.[16a–j]

SYNLETT 2010, No. 9, pp 1337–1340
Advanced online publication: 16.04.2010
DOI: 10.1055/s-0029-1219837; Art ID: S00510ST



Scheme 2 Synthesis of *S*-acyl glutathiones **3a–g**

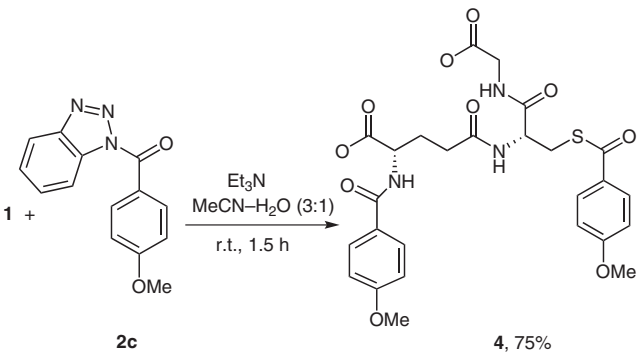
Table 1 Preparation of *S*-Acyl Glutathiones **3a–g**

RCOBt **2**	Product	Yield (%)	Mp (°C)
Fmoc-Val-Bt **2a**	**3a**	79	165–167
naproxen-Bt **2b**	**3b**	89	204–205
4-$MeOC_6H_4COBt$ **2c**	**3c**	95	222–224
4-MeC_6H_4COBt **2d**	**3d**	97	228–230
2-naphthylCOBt **2e**	**3e**	98	233–235
4-$O_2NC_6H_4COBt$ **2f**	**3f**	91	203–205
Cbz-Ala-Bt **2g**	**3g**	94	184–185

Treatment of GSH (**1**) with one equivalent of 1-acyl-1*H*-benzotriazoles **2a–g** in the presence of one equivalent of potassium bicarbonate at 20 °C in aqueous methanol for 15 minutes gave exclusively *S*-acyl glutathiones **3a–g** in 79–98% yields as the only products isolated (Scheme 2, Table 1). Of the two carboxylic acid groups present in the GSH, one forms a zwitterion with the amino group, but the other is free; we used potassium bicarbonate to neutralize it and then selectively S-acylated GSH.

Reaction of GSH (**1**) with two equivalents of 1-acyl-1*H*-benzotriazole **2c** in the presence of triethylamine at 20 °C in 1.5 hours gave the S,N-diacylation product **4** in 75% yield (Scheme 3).

In GSH, an anionic thiol group is more nucleophilic than an amino group, thus for selective N-acylation of GSH, we first protected thiol group of **1** with a 4-nitrobenzoyl



Scheme 3 Synthesis of bisacyl glutathione **4**

Table 2 Preparation of *S*,*N*-Diacyl Glutathiones **5a–e** and *N*-Acyl Glutathiones **6a–e**

RCOBt **2**	Yield (%) of **5**	Mp (°C)	Yield of **6** (%)	Mp (°C)
Cbz-Ala-Bt **2g**	**5a**, 84	132–134	**6a**, 65	182–183
Cbz-Phe-Bt **2h**	**5b**, 82	162–163	**6b**, 65	203–204
Cbz-Leu-Bt **2i**	**5c**, 89	150–152	**6c**, 78	198–200
4-$O_2NC_6H_4COBt$ **2f**	**5d**, 80	140–141	**6d**, 80	210–212
Fmoc-Val-Bt **2a**	**5e**, 84	183–184	**6e**, 63	174–175

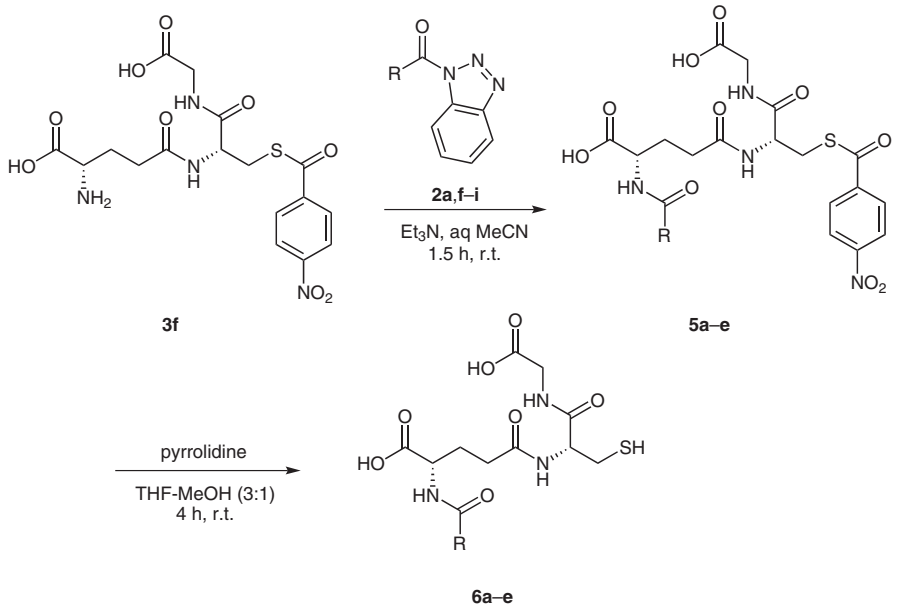
group. 4-Nitrobenzoyl is a known protecting group for thiols.[17] *S*-(4-Nitrobenzoyl) glutathione (**3f**) was synthesized by S-acylation of GSH (**1**, Scheme 2), **3f** was used an intermediate to make *N*-acyl glutathiones **6a–e**. *S*-(4-Nitrobenzoyl) glutathione (**3f**) was acylated with one equivalent of each of various 1-acyl-1*H*-benzotriazoles **2a**,**f–i** in the presence of one equivalent of triethylamine in aqueous acetonitrile for 1.5 hours to afford the corresponding S,N doubly acylated GSH derivatives **5a–e**, in 80–89% yields. Mild deprotection of the 4-nitrobenzoyl groups from these double acylated glutathione derivatives **5a–e** using pyrrolidine in dry THF–methanol for four hours at room temperature, gave the corresponding *N*-acyl glutathiones **6a–e** in 63–80% yields (Scheme 4, Table 2).

In conclusion, we have developed facile and efficient methods for selective syntheses for both *S*-acyl glutathiones[18] and *N*-acyl glutathiones[19,20] in good yields under mild reaction conditions using 1-acyl-1*H*-benzotriazoles. In comparison with literature methods, our methods comprise: (1) mild and simple reaction and workup conditions, (2) higher yields for the most of our products (65–98%), and (3) reduced reaction times (15 min to 6 h). These methodologies should have general applicability for the S-acylation and N-acylation of biologically active larger peptides and glycopeptides.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

Acknowledgment

We thank Dr. C. D. Hall for helpful discussions.



Scheme 4 Synthesis of *N*-acyl glutathiones **6a–e**

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(18) **General Procedure for the Preparation of Compounds 3a–e**

To a solution of GSH (1 equiv) in H_2O (3 mL), a solution of 1-acyl-1*H*-benzotriazole (1 equiv) in MeOH (8 mL) was added, and the mixture was stirred for 5 min at r.t. An aq $KHCO_3$ solution (2 equiv) in H_2O (1 mL) was added dropwise to the reaction mixture at a rate of 0.2 mL/min. The reaction progress was monitored by TLC (disappearance of 1-acyl-1*H*-benzotriazoles, which have R_f values in the range from 0.6–0.7 using solvent mixture of hexanes–EtOAc (2:1) as eluent; together with the appearance of benzotriazole at R_f value 0.45), which indicated the completion of the reaction within 10–15 min. MeOH was evaporated under reduced pressure and dilute HCl (6 M)/Na_2HPO_4 (1 N) was added until pH of the mixture was adjusted to 5. The precipitate formed was collected on a Buchner funnel, was washed with H_2O, EtOAc, and hexanes to afford the desired compound.

(19) **General Procedure for the Preparation of Compounds 5a–e**

A solution of 1-acyl-1*H*-benzotriazole (1 equiv) in MeCN (5 mL) and Et_3N (2 equiv) was added to a solution of **3f** (1 equiv) in MeCN–H_2O (1 mL – 2 drops). The reaction mixture was stirred at r.t. for 1.5 h. After completion of the reaction (by TLC as described above for compounds **3a–e**), the pH was adjusted to 3 using HCl (6 M). MeCN was evaporated under reduced pressure, and the resulting crude was triturated with Et_2O (5 mL) until a friable solid was formed. The solid was filtered and washed with additional

amount of Et_2O (2 mL) and dried to give the desired compound.

(20) **General Procedure for the Preparation of Compounds 6a–e**

Pyrrolidine (3 equiv) was added to a stirred solution of **5a–e** (1 equiv) in anhyd THF and MeOH (3:1, 4 mL). The mixture was stirred at 20 °C for 4 h. The solvents were then evaporated, and the resulting crude solid was dissolved in H_2O (2 mL) and MeOH (0.2 mL). The resulting solution was acidified to pH 1 using HCl (6 M) and stirred for 30 min. The precipitate was filtered and washed with Et_2O to afford the desired product.